University of California, Los Angeles
Office for Protection of Research Subjects
HUMAN SUBJECT PROTECTION COMMITTEE (HSPC)

APPLICATION TO INVOLVE HUMAN SUBJECTS IN RESEARCH

PROJECT TITLE:	HIV Replication and Thymopoiesis in Adolescents			
PRINCIPAL INVESTIGATOR:	Name: Paul Krogstad	Degree(s): M.D.	University Title: Associate Professor	Campus Phone No.: [redacted]
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CO-INVESTIGATOR or FACULTY SPONSOR:	Name: see attached list	Degree(s):	University Title:	Campus Phone No.:
	Department:	Campus Mailing Address:	Campus Mail Code:	e-mail Address:
PRIMARY CONTACT PERSON:	Name: Alison Watts	Campus Phone No.: {redacted]	e-mail Address: [redacted]	
APPLICATION STATUS:	**X** New ☐ Addendum ☐ Renewal	Previous HSPC number, if applicable:		

INVESTIGATOR'S ASSURANCE

I certify that the information provided in this application is complete and correct.

I understand that as Principal Investigator, I have ultimate responsibility for the conduct of the study, the ethical performance of the project, the protection of the rights and welfare of human subjects, and strict adherence to any stipulations imposed by the HSPC.

I agree to comply with all UCLA policies and procedures, as well as with all applicable federal, State, and local laws regarding the protection of human subjects in research, including, but not limited to, the following:

- performing the project by qualified personnel according to the approved protocol,
- implementing no changes in the approved protocol or consent form without prior HSPC approval (except in an emergency, if necessary to safeguard the well-being of human subjects),
- obtaining the legally effective informed consent from human subjects or their legally responsible representative, and using only the currently approved, stamped consent form with human subjects,
- **promptly reporting significant or untoward adverse effects to the HSPC in writing within 5 working days of occurrence.**
- if I will be unavailable to direct this research personally, as when on sabbatical leave or vacation, I will arrange for a co-investigator to assume direct responsibility in my absence. Either this person is named as a co-investigator in this application, or I will advise HSPC by letter, in advance of such arrangements.

_____
Principal Investigator Date

FACULTY SPONSOR'S ASSURANCE

By my signature as sponsor on this research application, I certify that the student or guest investigator is knowledgeable about the regulations and policies governing research with human subjects and has sufficient training and experience to conduct this particular study in accord with the approved protocol. In addition,

- I agree to meet with the investigator on a regular basis to monitor study progress.
- Should problems arise during the course of the study, I agree to be available, personally, to supervise the investigator in solving them.
- I assure that the investigator will promptly report significant or untoward adverse effects to the HSPC in writing within 5 working days of occurrence.
- If I will be unavailable, as when on sabbatical leave or vacation, I will arrange for an alternate faculty sponsor to assume responsibility during my absence, and I will advise the HSPC by letter of such arrangements.

_____
Faculty Sponsor ***** (if PI is a student or a fellow) Date

***** The faculty sponsor must be a member of the UCLA faculty. The faculty member is considered the responsible party for legal and ethical performance of the project.

SECTION II - FUNDING

THIS SECTION MUST BE COMPLETED

1. Check all of the appropriate boxes for funding sources for this research, include pending funding source(s):

   X Extramural* ☐ UCLA Academic Senate ☐ Department ☐ Gift ☐ Other: _____

   ***** P.I. of Contract or Grant: Paul Krogstad, M.D.

   Funding Source: National Institutes of Health

   Contract or Grant No.:

   Contract or Grant Title:

2. If using an **IDENTICAL** protocol for more than one extramural funding proposal, list all funding sources below. Attach an additional sheet if more space is needed.

   a. P.I. of Contract or Grant:

      Funding Source:

      Contract or Grant No.:

      Contract or Grant Title:

   b. P.I. of Contract or Grant:

      Funding Source:

      Contract or Grant No.:

      Contract or Grant Title:

3. **STATEMENT OF FINANCIAL INTERESTS:** If you are required to submit either a Form 730-U* or a Form 740-U* to the Office of Sponsored Research, please attach a copy of those form(s) with this application. See #9 of the Guidelines for additional information regarding this requirement.

   * Form 730-U, "Principal Investigator's Statement of Economic Interests" for non-governmental funded projects
   * Form 740-U, "Investigator's Statement of Financial Interests" for NSF or PHS funded projects

4. Is this application for the administrative approval for a training grant, a program project, a multiple project grant, or a center grants? ☐ Yes **X** No If yes,see Guidelines #14.

If this application is applying for an administrative approval for funding purposes only and does not involve the participation of human subjects, do not complete the rest of this application.

SECTION III - SUMMARY INFORMATION

THIS SECTION MUST BE COMPLETED

The review of research involving human subjects is conducted by either the Medical Human Subject Protection Committee (MHSPC) or the General Campus Human Subject Protection Committee (GCHSPC) depending on the nature of the protocol. The MHSPC is composed of primarily medical specialists, and the GCHSPC has principally socio-behavioral experts and some medical professionals. To aid the OPRS staff in evaluating which HSPC is most likely appropriate for the review of your protocol, please check all appropriate boxes in this section.

1. Will you perform medical procedures as part of this research proposal? XYes ☐No

2. **SUBJECT POPULATION:** (Check all appropriate boxes.)

X Children (*see Manual Chapters 4,6,8, & 10)*
☐Elderly (*see Manual Chapters 4 & 10)*
☐Fetuses (*see Manual Chapter 8)*
☐Pregnant women (*see Manual Chapter 8)*
☐Terminally ill (*see Manual Chapter 8)*
☐Comatose (*see Manual Chapter 4)*
☐Cancer patients (*see Guidelines #4)*
☐Cognitively or psychologically impaired (*see Manual Chapter 4)*
☐Institutional residents *(see Manual Chapters 4 & 8)*
☐Human in vitro fertilization (*see Manual Chapter 8)*
☐Exclusion of minorities (*see Manual Chapter 8)*
☐Prisoners or parolees (*see manual Chapter8)*
☐Non-English speaking (*see Guidelines #11 & Manual Chapter 8)*
☐UCLA students/staff (*see Guidelines #10 & Manual Chapter 8)*

3. If the research involves any of the following, check the appropriate boxes:

☐Interviews
☐Survey/questionnaire
☐Behavioral observation
☐Deception
☐Waiver of consent
☐Study of existing data *(see Guidelines #12)*
☐Study of human biological specimens *(see Guidelines #12)*
X Venipuncture (≤450cc)
X Genetic research
X HIV/AIDS
X Clinical studies
☐Investigational drugs *(if checked, complete Section V)*
☐Investigational devices *(if checked, complete Section VI)*
☐Radiation *(see Guidelines #5*)
☐Controlled substances *(see Guidelines #6)*
☐Microorganisms or recombinant DNA *(see Guidelines #7)*
☐Potential development of commercial product from human biological materials *(see Guidelines #8)*
X PI or Co-PI is the treating physician

4. **LOCATION(S) OF RESEARCH TO BE CONDUCTED AT:**

X UCLA campus
☐Santa Monica-UCLA Medical Center
X Other locations, specify: Children's Hospital, Los Angeles

5. **LAY LANGUAGE SUMMARY:** (Please use non-technical language that is understood by nonscientific members to summarize the proposed research project. The information must include: (1) a brief statement of the problem and related theory supporting the intent of the study, and (2) a brief but specific description of the procedure(s) involving the human subjects. Attach an additional page as necessary. However, please do not exceed one single-spaced, type-written page.)

The thymus is a large gland found in the chest. It is the source of CD4+ and CD8+ T cells, which play a key role in the body's defense against infection and cancer. These cells are progressively lost due to HIV infection. In people with HIV, treatment leads to an increase in T cells. It remains unclear how much of the increase is due to production of new T cells by the thymus and how much is due to the improved survival of existing T cells.

The intent of this study is to compare several aspects of the function of the thymus in subjects 13-24 years old who acquired HIV at birth versus subjects who acquired HIV through sexual activity or drug abuse versus subjects who are HIV negative. This will be done using blood collections as well as a CT scan of

the thymus. In addition, a substudy will be performed during which subjects will be admitted to the Clinical Research Center overnight. They will be given either a sugar solution or a water solution with a non-radioactive marker. Blood will be collected so that this marker can be detected.

SECTION IV - PROTOCOL SUMMARY

THIS SECTION MUST BE COMPLETED

INSTRUCTIONS: In order to review your proposal, the Human Subject Protection Committee (MHSPC) must have all of the following information. Each topic must be titled using the **boldface subheadings** listed below. State "Not Applicable" for topics that are not applicable to your application. Address each topic independently in the sequence listed without reliance on information covered under other subparts. Attaching sections of the grant application is not an acceptable substitute. Provide sufficient information for effective review by all members of the HSPC, including non-specialists. Define all abbreviations and terms not part of common language and use simple words and sentence structure as much as possible. Unless justification is provided, Section IV of this application must not exceed 10 pages (excluding references). Number each page, beginning with page one for the first page of Section IV.

INFORMATION REGARDING RENEWAL APPLICATION (1)

1. **Renewal Application:** What benefits to the participating subjects or to the society have been derived? Please also provide a summary of the research activities during the previous approval periods regarding the following issues:

   a) How many subjects have been enrolled since the date of last approval and since the initial approval?
   b) Has there been any difficulty obtaining/retaining subjects or obtaining informed consent during the previous approval period? If yes, describe:
      - Approximately how many potential subjects have refused participation?
      - How many subjects have voluntarily withdrawn participation at their own request?
      - How many subjects have withdrawn participation at the request of the PI?
   c) Have there been any unexpected reactions or complications since last scheduled annual review? If yes, please attach Adverse Event Reports (Form HS-5). If you have submitted the Adverse Event Reports, please state so.
   d) Approximately how many more subjects are required to complete the study?

   Not applicable – not a renewal

PURPOSE OF THE STUDY, THE BACKGROUND AND THE LITERATURE REVIEW (2-3)

2. **Purpose of the Study:** What are the specific scientific objectives (aims) of the research?

   1. To compare quantitative parameters of thymopoiesis from adolescents/young adults with perinatal HIV infection (PI-A) with those from age-matched seronegative control subjects (SN-A), and youth with HIV infection acquired via recent adult behaviors (AB-A).
   2. To evaluate the impact of viral factors on thymopoiesis of HIV infected adolescents.
   3. To examine the T cell receptor repertoire and CTL responses of perinatally infected adolescents.

3. **Background:** State the background of the study. Include a critical evaluation of existing knowledge, and specifically identify the information gaps which the project is intended to fill. Describe previous work in animal and/or human studies that provide a basis for the proposed research and that support the expectation of obtaining useful results without undue risk to human subjects.

   Note: Include appropriate citations to the scientific literature or attach a copy of literature review.

<u>Long term survival after HIV perinatal infection</u>.
Most cases of pediatric HIV-1 infection result from perinatal infection, occurring either *in utero* or at the time of delivery. Other cases are acquired postnatally via transfusion or breast-feeding. Regardless of the means by which

infection is acquired, untreated pediatric HIV infection is generally followed by the development of symptomatic disease in the first year of life, and the development of AIDS in as many as 50% of children by 5 years of age [1, 2] . In the pre-HAART era, the median and mean survival times for perinatally infected children were 8.0 and 9.4 years, respectively [2, 3] . However, survival into adolescence is now occurring in many cases. The number of these individuals is unclear, but the most recent HIV/AIDS Surveillance Report [4] notes 180 people who acquired HIV infection perinatally who have had AIDS diagnosed after age 13. It is likely that a much larger number of children were diagnosed with AIDS prior to age 13, and are now surviving into adolescence because of improvements in preventative care, and the advent of highly active antiretroviral therapy (HAART). In concert with the marked decrease in perinatal transmission seen in the last seven years, this increasingly large population of perinatally infected adolescents is changing the face of Pediatric HIV/AIDS in the United States.

Survival into adolescence is likely to be attributable to a combination of viral, host, and treatment factors. Chemokine and chemokine receptor gene polymorphisms, HLA type, and mutation of the viral *nef* gene are potentially important variables [5] . However, CCR5 deletions are uncommon in non-Caucasian populations that are most affected by the HIV epidemic [5, 6] , and large deletions in *nef* appear to be rare. There have been two small reports [7, 8] in which mutations of the *nef* gene were found in 8 long-term survivors of perinatal infection. In these reports, two patients had large *nef* gene deletions, and had strikingly mild disease. One was asymptomatic and on no antiretroviral therapy at 10 years of age, and did not have detectable plasma HIV RNA. The other was 12 years of age and had only moderately symptomatic disease (CDC class B). Missense mutations and small deletions of *nef* were found in some of the others. In contrast, most long-term surviving children have much more advanced disease than the subjects described in these reports. Nielsen et al [9] reported a multicenter study of pediatric long-term survivors, defined to be 8 or more years of age. Only 31/143 (21)% of children with maternally derived HIV infection and 9/54 (17%) with transfusion acquired infection had $\geq$ 500 CD4+ T cells and no prior AIDS defining conditions. Thus, in most cases, progressive immunodeficiency and AIDS are seen in adolescents who acquired HIV infection perinatally or via transfusion in early childhood, suggesting that these individuals are infected with fully pathogenic HIV variants.

Immunological changes associated with HAART.

The accumulated experience with HAART in adults has shown us that the prolonged suppression of HIV viremia to "undetectable" levels will often (though not invariably) arrest disease progression and bring about significant immunological restoration. Initially, the marked increase in CD4+ T cells seen with potent anti-retroviral agents was attributed to the reduction in virus-induced destruction or clearance of cells [10, 11] . Later reports made it clear that redistribution of lymphocytes from lymphoid organs accounted for much of the initial increase in peripheral blood T lymphocytes [12-14] . Subsequently, the qualitative and quantitative changes seen in response to HAART were better characterized. A triphasic pattern of immune reconstitution was described by Autran et al. in studies of adults. An early rise of memory CD4+ T cells was soon followed by improved T cell proliferation responses to recall antigens, and, finally, a late rise in putative naïve CD4+ T cells (CD45RA+ CD62L+). Functional improvements in cellular and humoral immune responses are often evident within months [15] . Similar patterns have been observed following HAART in children, although CD45RA+ T cells often increase in number during the first several months of therapy [16, 17] . Immunological improvements are less well documented during HAART in children, but include improved responses to measles immunization and proliferation responses to Candida [18-20] .

These data and the demonstration of decreased risk of opportunistic infections following prolonged HAART therapy [21, 22] have engendered optimism that suppression of HIV replication may be followed by at least partial restoration of normal immunity. However, it is clear that quantitative and functional immunological abnormalities generally persist. T cell counts remain below the normal range in most adults, and opportunistic infections continue to occur in some individuals. At a more subtle level, the T cell receptor repertoire continues to show marked perturbation even after long periods of successful HAART. This has been assessed in a number of ways, but is perhaps most readily quantifiable by using RT-PCR to examine the size distribution of sequences in the third coding complementarity determining region 3 (CDR 3) of the T cell receptor (TCR) chain. Insertion and removal of nucleotides during TCR recombination normally leads to a Gaussian distribution of the size of amplicons resulting from RT-PCR amplification of V mRNAs with primers that flank junction sites. Skewed distributions in CDR3 size pattern detected by this "spectratyping" are seen in chronic HIV infection, and often persist despite successful HAART [23-25] .

Persistent immunological defects may be more extreme following prolonged HIV infection in childhood. HIV infection in adults occurs after immunological development is complete, and substantial immunological experience with common pathogens has occurred. Perinatal HIV infection in children is characteristically is associated with high viral RNA levels throughout the first several years of life, perhaps leading to a disruption of immunological ontogeny, interfering with the acquisition of protective responses to CMV, toxoplasmosis, and other microorganisms [26, 27] . Moreover, HIV has been associated with accelerated thymic involution, which could be exacerbated by the sustained high-level viremia characteristic of early childhood. Indeed, an immunophenotype consistent with DiGeorge anomaly has been described in children with rapid progression of disease [28, 29] . The mechanism (s) underlying thymic injury by HIV are unclear, but the virus might directly kill thymocytes, or disrupt the microenvironment of thymic dendritic cells and epithelial cells required for normal thymopoiesis [30] . Although data from the SCID-hu mouse model indicate that the

thymic epithelial compartment may remain functional long after depletion of thymocytes has occurred [31] , there may be limits to this: age dependent difference in responses to HAART have been noted. Some studies indicate larger increases in CD4+ T cell counts are seen in younger compared to older adults [32] and in children compared to adults [33] . HAART may be less beneficial in perinatally infected adolescents, who have potentially had the longest exposure to injurious effects of HIV replication on the thymus.

Patterns and parameters of thymopoiesis.

The potential impact of perinatal HIV infection on the future survival of those who have survived into adolescence must be considered in light of recent developments in the understanding of thymopoiesis.

Until approximately twenty years ago, the thymus was thought to enlarge in childhood, and then involute during puberty under the influence of growth hormone and sex steroids. More recent studies of anatomy and physiology of the normal thymus have called this notion into question [30, 34] . Careful histological analysis of tissue revealed the thymus to be a chimeric organ containing epithelial tissue, where thymopoiesis occurs, and non-epithelial, perivascular space (PVS). In childhood, the thymic epithelial space (TES) decreases progressively, while the perivascular space gradually enlarges [35] . During adolescence, the total amount of lymphoid tissue remains constant, owing to an increase of the lymphoid PVS. Following adolescence, the PVS decreases, and fatty atrophy of the thymus is seen. However, after accounting for the perivascular space, Steinmann demonstrated a continuous loss of the lymphoid tissue in the cortex from 1 to 40 years of age [34] , without evidence of any change in the rate of loss during or after puberty. After the age of 40, the rate of involution of the TES decreases, but loss of the tissue involved in thymopoiesis continues. Still, the TES has been seen in centenarians, and recent reports have provided evidence of functional thymic activity in the fifth and sixth decades of life [36, 37] . While the thymus does not normally decrease in total volume with aging, HIV infection has been associated with a decrease in thymic volume, and histological evidence of premature atrophy, with decrease in TES, and an increase in the PVS. This effect may be a direct effect of HIV replication: HIV infected cells have been found in both the PVS and the TES [30] . The recent detection of infected naïve peripheral CD4 T cells is also consistent with this possibility [38, 39] .

If there is an effect of puberty on the rate of involution of the thymus, it does not appear to be readily detected in those not infected by HIV. However, delayed onset of puberty has been often seen in pediatric long-term survivors of HIV infection [40] , and castration prevents post-pubertal thymic involution in mice [41, 42] . It is conceivable that delayed puberty will have an effect on the rate of thymic involution of perinatally infected adolescents. If higher levels of testosterone and other androgens promote loss of functional tissue, delayed puberty may actually be beneficial, from a teleological perspective, in the face of ongoing HIV replication.

Until recently, non-invasive measures of the amount of functional thymus did not exist. An ideal laboratory method for the evaluation of HIV infected individuals would quantify both current thymic activity and the amount of healthy tissue that might persist.

Computed tomography has been used to examine the size of the thymus in adult lacking HHIV infections, corroborating post-mortem studies that found no change in the size of the thymus with age [43] . Clearly, radiographic imaging has significant limitations as a tool to evaluate the capacity for thymus dependent regeneration of T cell populations during HAART (or after cytotoxic chemotherapy or bone marrow transplantation). However, McCune and his collaborators found a correlation between thymic mass (any residual tissue not replaced by fat scored as an ordinal variable on a five point scale) and CD4 T cell number in HIV infected adults who were 20 to 59 years of age [44] . They also found a correlation between these estimates of the amount of thymic tissue, and increased thymopoiesis during HAART [45] . Similarly, Vigano et al used magnetic resonance imaging to monitor changes in thymic volume during HAART in a cohort of children with a mean age of 9.8 years at entry [46] . The thymus was markedly diminished in volume in HIV infected children with Class C disease. A rise in CD45RA+CD62L+ CD4+ T cells during HAART and was statistically associated with a change in thymic volume. Thus, the size of the thymus, as determined by radiographic imaging, has correlated with an increase in naïve T cells during HAART of both adults and children.

Until recently, no methods existed to identify and quantify lymphocytes that have recently emigrated from the thymus. In chickens, lymphocytes may be identified as recent thymic emigrants (RTE) with the monoclonal antibody chT1 [47, 48] . No comparable cell surface marker exists for humans or other mammals, but methods to quantify RTE have been developed based on the use of quantitative PCR measurements of circular DNA molecules generated during excisional rearrangement at the T cell receptor alpha [37, 49] or beta [50] chain loci. The PCR assay described by Douek et al [37] is used to quantify circular DNA molecules (termed signal joint TREC (sjTREC)) produced in approximately 70% of the cells with excision of the locus during maturation of thymocytes into T cells [51-53] . In control subjects, Douek et al found a nearly linear inverse correlation between age and TREC number in CD4+ and CD8+ T cells. These findings are in accord with the histological studies described above suggesting a gradual, progressive, diminution of the thymic epithelial space throughout the first four decades of life, without evidence of marked changes during puberty. In accord with these data, we have also found that TREC values are very stable in serial samples from HIV seronegative adolescents followed for up to 48 months (see preliminary studies below).

In agreement with several studies from adults, decreased TREC numbers are seen in chronically HIV infected children, and there appear to be age-dependent differences in responses to HAART: the increase in TREC seen with HAART

was greater in infants than in children with a median age of nine years [54]. Of note, Hazenberg et al have recently called the utility of TREC quantitation as a parameter of thymic output into question, noting that proliferation of naïve T cell populations may explain the drop in TREC seen after HIV infection has occurred [55]. However, this interpretation been called into question, as it is based on the measurement of TREC in measurement of cells identified as "naïve" by expression of CD27 and lacking CD45RO. As noted by Grossman and Paul, these markers would also be present in activated cells in transition to the expression of markers identifying them as memory T cells. For now, measurement of TREC appears to be a useful parameter of thymic output [56].

The *survival* of T cells produced during HAART could conceivably be influenced by the duration of previously uncontrolled HIV infection. Recent data suggest that maintenance of naïve and memory cells requires a supportive environment in the periphery, which may be distinct for each population [57]. While the signals necessary for the maintenance of the naïve T cells are still being defined, current data suggest that naïve CD4+ T cells are maintained without proliferation by contact with cells bearing MHC class II molecules [58]. Presumably, this represents the peripheral lymphoid mass, implying that HAART may need to bring about substantial normalization of lymph node architecture before immunological reconstitution is optimal. The chances for reconstruction of this microenvironment may be lower with longer periods of uncontrolled HIV replication after perinatal infection.

Directly measuring T cell dynamics in vivo may make it possible to determine if differences in T cell survival play a role in the clinical heterogeneity of long-term survivors of perinatal HIV infection. Hellerstein, McCune and their collaborators have shown that this is possible, by developing methods to determine rates of T cell turnover and cellular half-life [45, 59]. They administered prolonged infusions of glucose labeled with deuterium, a non–radioactive stable isotope, to uninfected and HIV-1 infected adults. Using gas chromatography-mass spectrometry, the fractional replacement of deuterium for hydrogen atoms was measured, allowing them to calculate the rate of turnover of T cells in the peripheral blood, and to estimate the half-lives of CD4 and CD8 T cells. In untreated HIV infected subjects, T cells had decreased half-lives, and no compensatory increase in T cell production. HIV treatment was associated with an increase in the absolute production rate of peripheral blood T cells, and normal half lives and production rates were restored after 12 to 36 months of HAART. In preliminary experiments performed with the assistance of Dr. Hellerstein, we have shown that these methods can be successfully applied to newborn macaques weighing *less than 1 kilogram.*

Deeks et al, have also recently helped explain the phenomenon of so-called "discordant responses" to HAART, in which higher CD4 T cell counts and peripheral blood TREC concentrations are measured in the face of high plasma HIV RNA concentrations [60]. This appears to be due to diminished fitness of the virus, resulting from *pol* gene mutations selected and maintained by the pressure of HAART. Circulating T cells exhibited near normal, despite measurable viremia. Removal of HAART was deleterious for the individuals they studied, who experienced a decrease in T cell counts within two to four weeks, which correlated with the re-emergence of circulating virus with increased susceptibility (decreased resistance) to reverse transcriptase and protease inhibitors. The destruction rate of CD4+ T cells increased in parallel (Deeks et al (JID in press)). They also demonstrated that HIV with mutations that confer decreased susceptibility to protease inhibitors also appeared to replicate poorly in thymic organ cultures. These data suggest that maintenance of a drug resistant genotype and phenotype may be an acceptable therapeutic goal for selected patients in whom HAART fails to control HIV replication. This is likely to be a fruitful area of investigation in the study of perinatally infected adolescents, who often have already been treated with a series of therapeutic regimens that failed to suppress HIV viremia.

Quantitation of CTL responses

The rate of progression of HIV related disease in untreated individuals is clearly related to the level of plasma viremia that becomes established after acute infection. By analogy to homeostatic physiologic mechanisms, this has been referred to as the viral "set-point", which appears to be linked to the magnitude of CD8 responses to HIV antigens [61]. Other studies involving adults have shown that slow progression of disease is correlated with the presence of potent CTL and CD4+ helper responses [15, 62-64].

Until recently, the mapping of CTL responses has been extremely labor intensive and technically challenging. Whole peripheral blood mononuclear cells (PBMC) from infected persons were randomly cloned at limiting dilution, followed by screening for HIV-specific cytolytic activity by chromium release using recombinant vaccinia. Alternatively, whole PBMC were specifically stimulated with recombinant vaccinia-infected autologous B cells (to enrich for HIV-1-specific CTL), then purified by limiting dilution cloning. More recently, the ELISpot assay has been increasingly used to map the breadth and magnitude of HIV-1 specific cellular immune responses [65, 66]. Fine-mapping of CTL responses is possible, due to the great sensitivity of this technique, which can identify cytokine release from individual cells. Because synthetic peptides slightly larger than the optimal epitope can be bound to class I MHC molecules when added exogenously, mapping of CTL responses within bulk uncloned PBMC can be accomplished using overlapping synthetic peptides covering the protein of interest.

This approach has been recently applied to the study of CTL responses in acute and chronic HIV. Goulder et al. used 290 overlapping peptides representing Gag, nef, RT, gp41, gp120, Tat and Rev , and 130 additional peptide represent previously known epitopes to map CTL responses in 11 subjects with early infection [66]. In two subjects, response to a common HLA-A*0201 restricted epitope were not initially detected, showing that these responses evolve, and

revealing the limitations of targeting mapping based on HLA type. These and other recent studies demonstrate that 1) cellular immune responses can evolve in a rapid fashion, 2) cannot be dependably predicted on the basis of MHC restriction [67] , and 3) can be thoroughly examined and quantified without necessarily knowing the amino acid sequences of autologous virus sequences.

Summary.

Detailed studies of the interplay between HIV infection, thymic output (thymopoiesis), and antiretroviral therapy, have been critical to recent advances in the understanding of the pathogenesis of HIV infection and AIDS. The urgency to better understand the effects of HIV on thymopoiesis is evident when we consider that HAART has made survival into adulthood quite likely for the majority of infected infants and children in the United States and other countries where intensive antiretroviral therapy is available and accessible. Comprehensive studies of thymopoiesis, and characteristics of HIV that impair it may guide our efforts to preserve and improved immunological functions in HIV infected adolescents. PCR methods to quantify recent thymic emigrants, and *in vivo* labeling methods to track the fate of these cells, have made it possible to more comprehensively examine the mechanistic underpinnings of T cell depletion in pediatric HIV infection. For the first time, methods now exist to quantify with some accuracy the production, function, and clearance of T cells.

We do not know what combination of viral, host, and treatment factors has allowed some infants to survive into adolescence after perinatal HIV infection, and can presently only speculate what the future holds in store for them. Taking scientific advantage of the large number of perinatally infected adolescents and young adults in the Los Angeles area, we are proposing studies to examine the balance between the pathogenic properties of HIV, the suppressive and selective power of antiretroviral therapy, and the regenerative capacity of the immune system that exists in these individuals.

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CHARACTERISTICS OF THE SUBJECT POPULATION (4-6)

4. **Number of Subjects:** What is the anticipated number of subjects to be enrolled at UCLA and, in the case of multi-center research, the total number of subjects for the entire project?
A total of 60-90 adolescents and young adults will be enrolled. There will be 20-30 subjects from each group (perinatally infected = PI-A; seronegative = SN-A; and adult behavior infected = AB-A).

5. **Inclusion/Exclusion Criteria:**
   a) What are the criteria for inclusion and exclusion?
   b) How will eligibility be determined, and by whom?
   c) Are any inclusion or exclusion criteria based on age, gender, pregnancy or childbearing potential, or racial/ethnic origin? If so, explain and justify.

   Note: Equitable inclusion of both men and women of all ages, and individuals from diverse racial/ethnic backgrounds, is important to assure that they receive an equal share of the benefits of research and that they do not bear a disproportionate share of its burdens. Participation of adult subjects of both genders and diverse racial/ethnic backgrounds should not be restricted without medical or scientific justification.

   a.
   The study is open to subjects between the ages of 13 and 21 at the time of enrollment, irrespective of race and gender. Given the potential 30 month study period, all subjects will complete the study at the age of 24 or younger. 20-30 HIV positive subjects, perinatally infected subjects will be recruited, along with 20-30 subjects seronegative for HIV and 20-30 HIV positive subjects who acquired HIV through adult behavior.
   b.
   Subject eligibility will be determined by the principal investigator based on the above criteria
   c.
   Subjects 12 and younger will be excluded since this study will focus on HIV pathogenesis in pubertal and post pubertal children. Subjects greater than 21 at the time of screening will be excluded for the same reason.

6. **Vulnerable Subjects:** Will any vulnerable subjects be included? If so, identify the subject groups and justify their involvement.

   Examples of vulnerable subjects: children, elderly, pregnant women, fetuses, cognitively impaired individuals, persons with severe psychological disorders, terminally ill patients, emergency patients, institutional residents, prisoners, parolees, non-English speaking subjects, and UCLA students/staff.

   Adolescents and young adults will be the sole subjects, since the study is designed to focus on HIV pathogenesis in pubertal and post pubertal children.

SUBJECT IDENTIFICATION AND RECRUITMENT (7)

7. **Method of Subject Identification and Recruitment:** What method(s) will be used to identify and recruit prospective subjects? Attach a copy of any planned advertisements/notices and letters to potential subjects.

   Note: The identification and recruitment of subjects must be ethically and legally acceptable and free of coercion. Procedures used to recruit subjects should be designed to reach diverse populations. Vulnerable subjects, such as persons in nursing homes or institutions, should not be recruited merely for the sake of convenience.

   Subjects will be recruited through the use of flyers (see attached). In addition, the investigator will let his colleagues within UCLA know about the existence of the study and will give them flyers such that interested subjects may contact the investigator to learn more about the study.

METHODS AND PROCEDURES (8-11)

8. **Methods and Procedures Applied to Human Subjects:** Describe the study design and all procedures (sequentially) to which human participants will be subjected. Identify all procedures that are considered experimental and/or procedures performed exclusively for research purposes.

   Note: A clinical research protocol may involve interventions that are strictly experimental or it may involve some aspect of research (e.g., randomization among standard treatments for collection and analysis of routine clinical data for research purposes). It is important for this section to distinguish between interventions that are experimental and/or carried out for research purposes versus those procedures that are considered standard therapy. In addition, routine procedures performed solely for research purposes (e.g., additional diagnostic/follow-up tests) should be identified.

   Month 0
   After consenting/assenting to participate in the study, subjects will have a physical exam and will have blood collected. They will also have a CT scan.

   Month 6
   Subjects will have a physical exam and will have blood collected. They may also be asked if they want to participate in the substudy (see below).

   Months 12 and 18
   Control (non HIV subjects) will have a physical exam and will have blood collected. The study ends for control subjects at month 18
   HIV infected subjects will have a physical exam and will have blood collected. Depending on the results of their month 12 blood work, they may have a CT scan at the month 18 visit.

   Months 24 and 30
   Only HIV infected subjects who have changed their anti-HIV medication regimen will have visits at month 24 and month 30. At these visits, they will have blood collected, a physical exam, and a may have CT scan.

   Substudy
   At month 6, subjects may be asked if they would like to pariticipate in the substudy. If they are interested, they will consented/assented using a separate consent/assent. Subjects would be admitted to the GCRC. They will be infused with a deuterium labeled glucose over a 24 hour period. A fingerstick for glucose monitoring will be done at 12 and 24 hours. The subject will be asked to return to the clinic between 4 and 7 days, and then again between 8 and 14 days for blood collection (up to 50 ml at each visit, but dependent on body weight). It is uncertain if this glucose infusion will allow adequate labeling. If it does not, subsequent subjects would be switched to receive 70% D_2O over 24 hours. They will then be sent home with pre-measured aliquots of D_2O to drink 2-3 times per week, and they will be asked to return to the clinic for blood samples at days 14 and days 28 (up to 50 ml at each visit, but dependent on body weight)

9. **For Research Involving Survey, Questionnaires, etc.:** Describe the setting and mode of administering the instrument (e.g., by telephone, one-on-one, or group) and the provisions for maintaining privacy and confidentiality. Include the duration, intervals of administration, and overall length of participation.

   Note: If the protocol for the interviews or the questionnaires are not yet designed, provide a sample of the questions or describe the subject matter to be covered. (If the instrument has been prepared even in draft form, submit a copy.) The final survey instruments or questionnaires must be reviewed and approved by the HSPC before they may be used.

   Not applicable

10. **FDA Approval:** Are there any investigational drugs or biological agents used in this study? If yes, please complete Section IV. Are there any investigational devices used in this study? If yes, please complete Section V. If the study does not involve any investigational drugs or devices, this should be stated.

Not applicable

11. **Data Collection, Storage and Confidentiality:**
    a) How will data be collected and recorded? Will it be associated with personal identifiers or coded to protect personal privacy?
    b) Where will the data be stored during the study and how will it be secured?
    c) Who will have access to the data and/or to the codes? If data with subject identifiers will be released, specify the person(s) or agency to whom this information will be released.
    d) What will happen to the data when the research has been completed?

    Note: The principal investigator is responsible for taking all necessary steps to maintain confidentiality of data. This includes coding data and choosing an appropriate and secure data storage mechanism that will prevent unauthorized access to the data. Where appropriate, the principal investigator should seek a certificate of confidentiality from the federal government.

    a. Data will be collected and recorded on forms designed by the study team. These forms will be coded to protect personal privacy.
    b. All data will be kept confidential and will be stored in locked storage in the office of the investigator.
    c. Only the investigator and his study team will have access to the data.
    d. The data will be stored by the principal investigator.

RISK/BENEFIT ASSESSMENT (12-17)

12. **Potential Risks and Discomforts:** What are the potential risks/discomforts associated with each intervention or research procedure? If data are available, estimate (a) the probability that a given harm may occur, (b) its severity, and (c) its potential reversibility.

    Note: A risk/discomfort is a potential harm associated with the research that a reasonable person would consider important in deciding whether to participate in the research. Risks can be generally categorized as physical, psychological, sociological, economic and legal.

    <u>CT scan</u>
    Subjects will be exposed to a small amount of radiation from the CT scans. The number of CT scans will be kept to a minimum, with repeat scans done only on HIV infected subjects who have changed their HIV medications or whose blood work indicates changes in their immune status.

    <u>Blood collection</u>
    Pain, bruising, rarely infection or fainting may occur

    Additional risks of Substudy:

    <u>Infusion of labeled glucose</u>
    Pain, bruising, rarely infection or fainting may occur

    <u>Overnight stay in GCRC</u>
    The stay may cause inconvenience to the subject and his or her family.

13. **Risk Classification:** What is the overall risk classification of the research: minimal, greater than minimal, significant, or unknown?

    Note: According to HHS/FDA Regulations minimal risk means "The probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests." When the risks associated with a new procedure or product are unknown, they cannot be classified as "minimal." Your estimation of risk determines the Emergency Care and Compensation for research-related Injury clause in the informed consent form.

Greater than minimal

14. **Minimizing Risks:** What procedure(s) will be utilized to prevent/minimize any potential risks or discomfort?

    Note: All potential risks and discomfort must be minimized to the greatest extent possible by using procedures such as appropriate monitoring and withdrawal of the subject upon evidence of a specific adverse event or clinical sign(s). This section should reflect that all appropriate steps will be taken to protect subjects from harm.

    All procedures will be performed by trained medical personnel. CT scans after the month 0 visit will be done only on subjects who have altered their HIV medications or whose blood work shows changes in their immune status. Blood volumes collected will be based on subject's body weight to ensure the guidelines regarding maximum collection are not exceeded.

15. **Potential Benefits:**
    a) What potential benefits may subjects receive as a result of their participation in the research?
    b) What potential benefits to society may be expected from this research?

    Note: Societal benefits generally refer to the advancement of medical knowledge and/or possible benefit to future patients

    a. Subjects will not benefit from their participation in this study.
    b. The study results may give more information on the affects of HIV infection on the immune status and the thymus, leading to better treatments for HIV infection.

16. **Therapeutic Alternatives:** What therapeutic alternatives are reasonably available in the non-research and/or research context that may be of benefit to the potential subjects?

    Note: This section should include a reasonably detailed description of the therapeutic alternatives that could be used to treat the patient should they elect not to participate in the protocol.

    Not applicable – the study is not designed to provide therapeutic intervention. The alternative to participation is not to participate.

17. **Risk/Benefit Ratio:** What is the risk/benefit ratio of the research, compared with that of the available alternatives?

    Note: The potential benefits of research must justify the risks to human subjects. Some risks may not be reasonable, no matter how important the potential benefits. The risk/benefit ratio of the research must be at least as favorable for the subjects as that presented by standard treatments for their condition. When comparing the risk/benefit ratio of research with that of available alternatives, the alternative of doing nothing, or "watchful waiting," should be included in the analysis.

    The risks of the study are minimal. Blood collection and CT scans are routine procedures that present minimal risk to the subjects. The pathogenesis of HIV infection and its affect on the thymus through puberty and immediately post puberty are not well understood. This study may give valuable information regarding this, thus potentially leading to more effective treatments. Thus we feel that the benefits outweigh the risks.

FINANCIAL CONSIDERATIONS (18-20)

18. **Payment for Participation:** Describe all plans to pay subjects, in cash or in kind. If no payment is planned, that should be stated. Information regarding payment consideration should include: Will subjects receive any financial inducement or payment for participation? Will they receive services or other benefits instead of cash? Will they be reimbursed for travel and other expenses? What conditions must be fulfilled by subjects to receive either full or partial payment?

Note: The FDA encourages a prorated system of payment whereby subjects who do not finish the protocol are paid in proportion to the part completed. The amount of payment must be justified and not constitute undue inducement of the subject to participate in the research. If a non-prorated system of payment will be used, this should be justified in this section.

Control subjects will be paid $20 per visit for the main study. HIV infected subjects will be paid $10 per visit for the main study. This is less than the control subjects, since the HIV subjects will likely have clinical visits for routine care at the same frequency as the study visits, and we will attempt to perform the study visits in conjuction with the clinic visits to maximize convenience for the subjects. For the substudy, subjects will be paid $75 for the overnight visit, $25 for the first visit for blood collection, and $50 for the second visit for blood collection.

19. **Financial Obligations of the Subjects:** What financial obligations will subjects incur as a result of participating in the study? Will subjects have to pay for any of the treatment(s) they receive or tests performed in the research?

    Note: This section should clarify who will pay for procedures associated with the study as well as financial responsibility for routine clinical care (e.g., Diagnostic tests, hospitalization, follow-up). Insurance and other third party payers may not cover procedures associated with participation in research (even if they might have paid for some of the procedures in connection with standard therapy). Consequently, subjects' costs may be increased as a result of additional follow-up examinations and/or tests required by the research.

    There will be no charge to the subjects for participation in this study. Subjects will not be charged for their office visits, physical exams, blood draws, lab work, CT scans, overnight stay in the GCRC, the labeled glucose or water.

20. **Emergency Care and Compensation for Research-Related Injury:** If the research presents greater than minimal risk, what emergency care is available in case of research-related injury? Who will be responsible for the cost of such care? Will subjects be compensated for out-of-pocket expenses or lost wages if they suffer a research-related injury?

    Note: Standard language for explaining this to prospective subjects is provided in the instructions for preparing the Consent Form. (Forms HS-2 & HS-3).

    Subjects who are injured as a result of research procedures not done primarily for their own benefit will receive treatment at no cost.

INFORMED CONSENT (21-26)

21. **Capacity to Consent:** Will all adult subjects have the capacity to give informed consent? If not, describe the likely range of impairment and explain how, and by whom, their capacity to consent will be determined.

    Note: In research involving more than minimal risk, capacity to consent should be determined by a psychiatrist, clinical psychologist, or other qualified professional not otherwise involved in the research. Individuals who lack the capacity to consent may participate in research only if consent is given on their behalf by a legally authorized representative.

    Subjects 18 and older will be given the consent form. Subjects younger than 18 will be given the youth assent form and their parents will be given the consent form. All subjects will have the capacity to consent/assent

22. **Personnel Inviting Participants:** Who will be inviting subjects to participate and what will they say? Identify by name and training the individual(s) authorized to describe the research to subjects/representatives and to invite their participation.

Note: Only those individuals authorized to solicit consent may sign the consent form confirming that the prospective subject was provided the necessary information and that any questions asked were answered.

The investigator will invite all subjects to participate and will invite the parents/guardians to consent for adolescents 13-17 years old. He will describe the study to the subjects and will reiterate that their participation is completely voluntary.

23. **Process of Consent:** How and where will the consent process take place? How will it be structured to enhance independent and thoughtful decision-making? What steps will be taken to avoid coercion or undue influence?

Note: Consider: a) the environment and location where informed consent will be solicited; b) the timing of the process (e.g., in relation to hospital admission, surgery, medication, stressful events); c) the involvement of someone other than the investigators to help explain the research; and d) opportunity for the prospective subjects/representatives to discuss participation in the research with family, friends, or their advisors before signing the consent form.

The consent will be obtained immediately after the subject determines that s/he is interested in possible participation in the study. The investigator will review the consent/assent form with the subject and the consent form with the parent/guardian as applicable. The subject and parent/guardian will be given ample opportunities to ask questions and will be questioned to ensure his/her understanding of the information in the consent/assent form. The subject will be encouraged to take the consent/assent form home with him/her to review it with family/friends. The subject will be told that his/her participation in the study is voluntary and will be reassured that s/he may elect not to participate without affecting his/her relationship with UCLA or with the investigator. The subject will be told that his/her participation will in no way affect the relationship with the physician or the treatment that the subject will receive from the physician. The alternatives to participating in the study will be reviewed with the subject. The consent /assent will be obtained in a private room.

24. **Comprehension of the Information Provided:** How--and by whom--will it be determined whether the subjects or their legally authorized representatives understand the information provided?

Note: This section should clearly document that the investigator has an adequate plan in place to assure existence of an acceptable level of comprehension before consent is documented. The principal investigator (or approved designee) is responsible for assuring that prospective subjects or their representatives have sufficient understanding of the research to make an informed decision about participation. It is important that they understand the purpose of the research, the nature and duration of the procedures, any risks and discomforts involved, the possible benefits to the subjects and others, and their right to withdraw consent at any time without penalty. Willingness to sign the consent form is not an adequate demonstration of their understanding. Some investigators try to determine the level of prospective subjects' comprehension by questioning them about the research. (This approach is useful with children and adolescents, as well as with adults of uncertain capacity to consent.)

The potential subject will be asked by the investigator whether s/he understood the information in the consent/assent form. The potential subject will be asked questions about information in the consent/assent form to ensure understanding. The investigator will review information in the consent form with the subject to ensure comprehension of the requirements of the study and of each study visit. The subject will be questioned to ensure that s/he understands the risks associated with the study, the potential benefits, and the alternatives to participation in the study. Parents/guardians will be asked the same type of questions.

25. **Information Withheld From Subjects:** Will any information about the research purpose and design be withheld from potential or participating subjects? if so, explain and justify the non-disclosure and describe plans for post-study debriefing.

Note: Any non-disclosure must be approved by the HSPC and may not exclude information that a reasonable person would want to know in deciding whether to participate in the research. In addition, the alteration in the consent procedure must be approvable under 45 CFR 46.116(d): (1) the

research involves no more than minimal risk to the subjects; (2) the waiver or alteration will not adversely affect the rights and welfare of the subjects; (3) the research could not practicably be carried out without the waiver or alternation; and (4) whenever appropriate, the subjects will be provided with additional pertinent information after participation.

No information will be withheld

26. **Consent/Assent Forms:** Specify the form(s) that will be used among the following: adult consent form, parental consent form, proxy consent form, youth assent form (age 13-18), and/or child assent form (age 7-12).

    Subjects 18 years of age and older will use the adult consent form. Subjects 13-17 will use the youth assent form. Parents/guardians of subjects 13-17 will be given the adult consent form.